end of IL7/placebo treatment was higher in the IL7-treated animals (122 vs 1/ml, p=0.03). All animals were pretransplant cytomegalovirus seropositive. One animal died at the end of IL7 treatment; necropsy showed extensive T cell infiltration of kidneys and lungs. In conclusion, IL7 stimulates the expansion of CD4 T cells, including functional anti-viral cells. Clinical risk:benefit ratio needs to be evaluated.

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DE NOVO GENERATION OF CD4 T CELLS AGAINST VIRUSES PRESENT IN THE HOST DURING IMMUNE RECONSTITUTION

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Patients after hematopoietic cell transplantation lack naïve T cells because of insufficient generation of T cells *de novo* (thymopoiesis). Ways to improve *de novo* generation have recently been studied, using, e.g., prothymopoietic cytokines or a thymic organoid ("artificial thymus"). It is not known whether improved thymopoiesis might result in more T cells only for pathogens that are typically absent from the body (e.g., neopathogens like ebola virus or cleared recall pathogens like measles virus) or also for pathogens or malignant cells present in the body (e.g., herpesviruses that infected the host pretransplant or the leukemia that necessitated hematopoietic cell transplantation). The latter T cells may be more important, as posttransplant infections due to endogenous microorganisms (e.g., herpesviruses) and leukemic relapse cause major morbidity and mortality. However, T cells recognizing self-peptides should theoretically be deleted in the thymus by negative selection. Peptides from the endogenous viruses or leukemic cells might be considered by the thymus as self-peptides, and T cells specific for these peptides might be deleted (negatively selected). We demonstrated in four baboons infected with baboon cytomegalovirus and baboon lymphocryptovirus (Epstein-Barr virus-like virus) that after autologous transplantation of yellow fluorescent protein (YFP)-marked hematopoietic cells, YFP^+ CD4 T cells against these viruses were generated *de novo*. Thus the thymus generates CD4 T cells against not only pathogens absent from the host but also pathogens present in the host. This finding provides a strong rationale to improve thymopoiesis in hematopoietic cell transplant recipients.

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CROSS-REACTIVITY OF EPSTEIN BARR VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES WITH A HLA-C LOCUS ALLELE

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The basis for T cell alloreactivity is thought to be primarily due to cross-reactivity of antigen-specific T cells with Major Histocompatibility Complex alleles. The cross-reactivity of cytotoxic T lymphocytes (CTL) from $HLA\text{-}B8^+$ individuals recognizing the human herpesvirus IV (HHV4/EBV) FLRGRAYGL epitope encoded by the EBNA-3A latency antigen with self peptides presented on HLA-B4402, B35, and B14 are examples of this phenomenon and may represent the mechanism by which herpes virus infections augment graft vs host disease, allograft rejection and autoimmunity. Here we describe an EBV-specific CTL line prepared from a HLA-B8-negative haplo-identical parental hematopoietic progenitor cell donor to restore patient immunity to EBV, that reacts strongly to non-EBV infected targets from the intended recipient. The CTL line was primed 2× with donor-derived EBV-transformed B lymphoblastoid cells lines (BLCL) at a responder: stimulator ratio of 40:1 that was reduced to 4:1 at wk 3 with the addition of IL-2 to the culture and was expanded to wk 5 at which time it was tested for reactivity to donor BLCL and donor and patient PHA blasts. The line showed potent reactivity to donor BLCL (66.3% specific lysis at an effector:target ratio of 50:1), no reactivity donor PHA blasts but unexpectedly, lysed patient PHA blasts (41.6% at 50:1) in repeated assays. Screening of the line with PHA blasts from individuals sharing one or two HLA alleles on the mismatched patient haplotype revealed that lysis was directed to HLA-C*0202. Screening of the EBV-CTL line with BLCL targets from unrelated donors who shared donor HLA alleles but lacked C*0202 showed that the line was partially HLA-restricted by HLA-DQ-*0201, B*3701, B*0702, and C*0701. A new line from the same donor primed for only two weeks with autologous BLCL at a responder:stimulator ratio of 4:1 also showed strong reactivity to donor BLCL and patient C*0202+ PHA blasts. T cells producing IFN-γ in response to irradiated C*0202+ PHA blasts were selected from this line using a MACS column. Further characterization of the expanded MACS-selected CTL for EBV specific antigen killing and HLA restriction is in progress. These data further support cross-reactivity to peptide presented on self MHC as the basis for T cell alloreactivity and illustrate the need for careful screening of allo-EBV-CTL from HLA mismatched donors with non-EBV infected patient targets to ensure that these lines are safe to infuse into patients.

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CTL ACTIVITY OF *IN VITRO*-DERIVED MEMORY T CELLS IS MORE EFFICIENT THAN NAIVE T CELLS IN SYNGENEIC RECIPIENTS IMMEDIATELY FOLLOWING EXPERIMENTAL BMT

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We are interested in elucidating how transplanted memory CD8 T cells can contribute to the immune reconstitution of ablatively conditioned BMT recipients. We have focused our studies on memory CD8 T cells because they display more rapid, elevated cytotoxicity compared to naïve CD8 cells, and thus may make more efficient BMT adjuvants. A homogeneous TCR-transgenic (tg) memory CD8 T population was generated *in vitro* and used in a syngeneic murine BMT model to 1) investigate the homeostatic proliferation and function of *in vitro*-derived memory T cells and 2) compare the CTL activity of these memory cells to that of naïve T cells in the reconstituting host. The *in vitro*-derived memory T cell population (TM) was generated using either OT.I-$RAG1^{-/-}$ or OT.I-GFP TCR tg mice. Briefly, spleens from OT.I mice were cultured for 3 days with rmIL-2 and OVA peptide, harvested, and re-cultured for 2–4 days with rmIL-15. The resultant OT.I cells expressed $CD25^{lo}$, $CD44^{hi}$, $CD62L^+$, and $Ly6C^+$, indicating a central memory phenotype. The OT.I cells (1.5×10^6), together with T cell-depleted B6 bone marrow (2×10^6), were transplanted into 9.0 Gy-conditioned syngeneic recipients. Homeostatic proliferation of TM was assessed by $V\beta5^+$, GFP^+ cell numbers in recipient spleens 4 to 42 days post-BMT. TM expanded during the first 14 days (2×10^6/spleen) then maintained its number while the spleen continued to increase for another two weeks. TM cell number and phenotype remained unchanged >90 days post-BMT. CFSE-labeled OT.I-$RAG1^{-/-}$ cells were used to precisely examine when, during the first 2 weeks post-BMT, TM underwent division. The first several divisions were apparent as early as 3 days post-BMT, and the TM population, as a whole, continued to divide with 6 divisions observed by day 7. CTL function was assessed within 14 days and then >28 days post-BMT using OVA-pulsed targets. TM obtained from recipient spleens 7 days post-BMT generated a more rapid, elevated CTL response compared to transplanted naïve OT.I cells ($p < 0.05$). TM remained functional at least 5 weeks post-BMT. In total, these findings demonstrate that a homogeneous *in vitro*-derived memory CD8 T cell population can efficiently expand and function immediately post-BMT. Moreover, these memory CD8 T cells exhibit greater cytotoxic activity compared to naïve CD8 T cells, supporting the notion that rapidly-generated, *in vitro*-derived memory CD8 T cells may have the superior therapeutic potential immediately following BMT.

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RECONSTITUTION OF IMMUNITY TO ADENOVIRUS AFTER PEDIATRIC BONE MARROW TRANSPLANT

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Adenovirus has been shown to be a significant cause of morbidity, and mortality in the pediatric hematopoietic stem cell transplant